43551

National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Division
Division des thèses canadiennes

Ottawa, Canada
K1A 0N4

PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

• Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur

SINCLAIR, LYNN JANETTA

Date of Birth — Date de naissance	Country of Birth — Lieu de naissance
FEBRUARY 9, 1951	CANADA

Permanent Address — Résidence fixe

60 AINSDALE ROAD
SCARBOROUGH, ONTARIO
M1R-3Z2

Title of Thesis — Titre de la thèse

University — Université

UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

MASTER OF SCIENCE

Year this degree conferred — Année d'obtention de ce grade	Name of Supervisor — Nom du directeur de thèse
1979	HOWARD A. WENGER

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

Date	Signature
OCTOBER 30, 1979	L Sinclair

NL-91 (4/77)

National Library of Canada

Cataloguing Branch
Canadian Theses Division

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada

Direction du catalogage
Division des thèses canadiennes

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE UNIVERSITY OF ALBERTA

EFFECTS OF AGE AND PROLONGED PHYSICAL CONDITIONING ON COLLAGEN IN ACHILLES TENDON OF YOUNG AND ADULT RATS

by

(C)

LYNN J. SINCLAIR

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF PHYSICAL EDUCATION

EDMONTON, ALBERTA

Fall, 1979

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled EFFECTS OF AGE AND PROLONGED PHYSICAL CONDITIONING ON COLLAGEN IN ACHILLES TENDON OF YOUNG AND ADULT RATS, submitted by Lynn Janetta Sinclair in partial fulfilment of the requirements for the degree of Master of Science in Physical Education.

.....
Supervisor

.....

.....

Date June 18, 1978

APPRECIATION

This thesis is just a small part of a more total education and experience afforded me by THE UNIVERSITY OF ALBERTA, Physical Education professors and fellow graduate students. I extend my warmest appreciation to all these people, and....

To my advisor, Dr. Howie Wenger, an exceptional person professionally and personally, may I say what an honour it was for me to be both student and friend

To my committee members, Dr. Ted Wall and especially to Dr. David Secord and the laboratory staff under the direction of Moira at the Surgical Medical Research Institute for giving so freely of their time, knowledge and technical facilities necessary for my research

To John Wilkinson, Norm Gionet and Dave Wiles for sharing those many hours together in the lab

To Marika Sesztak, my typist, a sincere thank you

To Jim Sexsmith, Jane Gardner, Heather Hartsell, Jan Raymond and Jim Donlevy who saw me through to the end

To John Barry, my long-time friend, without whose influence I would never have come to Edmonton.

DEDICATION

To Mom and Dad

ABSTRACT

The purpose of the study was to investigate the effects of age and sedentary, moderate (endurance) and intensive (sprint) physical conditioning on body weights and chemical changes in collagen of achilles tendons during growth and maturation of rats. Physico-chemical changes in collagen as indicated by hydroxyproline concentrations (ug/mg) (0.45 M NaCl extraction) were determined in order to permit physiological age comparisons of active and non-active groups.

Ninety male albino rats of Wistar strain at 5 ± 1 weeks of age were exposed to a sedentary control condition (CS), continuous endurance-type running (EC), and sprint-type interval running (SI) and studied at 15, 25, 32 weeks of age. EC and SI conditioned animals were progressively accommodated to a Quinton rodent treadmill at 35 m/ min/ 60 min/ day, 8% grade and 85 m/ min/ 20 bouts/ day (1 bout 15 s : 20 s), 30% grade respectively, 4 days per week for durations of 10, 15, 26 weeks. SI - 32 week old group was further accommodated to running speeds of 90 m/ min/ 20 bouts/ day, 30% grade.

Significant ($p < 0.05$) increases of body weight occurred with growth (15 and 25 weeks). Endurance conditioned rats had significantly lower body weights compared to sedentary, free eating controls. Total collagen content as determined by hydroxyproline concentrations (ug/mg stored weight) increased as a function of age (15 and 32 weeks). The present study suggests that no conclusive evidence exists to substantiate the notion that there is a relationship between physical conditioning and aging in collagen of achilles tendons of 15, 25, 32 week old rats.

TABLE OF CONTENTS

SECTION

PAGE

INTRODUCTION 1

Definition of Terms 7

METHODOLOGY 9

Experimental Design 9

Animal Care and Laboratory Conditions 11

Attrition 11

Training Protocol 11

- Endurance Training Mode 12

- Sprint Training Mode 14

Tissue Collection, Preparation and Analysis 14

Statistical Analysis 17

RESULTS 18

DISCUSSION 21

Conclusion and Recommendations 27

***

REFERENCES 28

APPENDICES

A. REVIEW OF LITERATURE 34

B. LABORATORY TECHNIQUE FOR HYDROXYPROLINE DETERMINATION 40

C. STATISTICAL ANALYSES 46

Scheffé Post Hoc 47

LIST OF TABLES

Table	Description	Page
I	Experimental Design, Experimental Unit Nomenclature and Sample Size for Age (wks) (Factor A) and Activity Pattern (Factor B)	10
II	Computed Cell Means Matrix of Total Collagen Content as Indicated by Hydroxyproline Concentrations (ug/mg) of Achilles Tendon as a Function of Age(wks) and Activity Pattern in Young and Adult Male Rats	23
III	Computed Cell Means Matrix of Insoluble Fraction of Collagen as Indicated by Hydroxyproline Concentrations (ug/mg) in 0.45 M NaCl from Achilles Tendon as a Function of Age (wks) and Activity Pattern in Young and Adult Male Rats	26
IV	Current Studies Investigating the Effects of Physical Conditioning and Age on Collagen Content as Determined by Hydroxyproline Analyses of Connective Tissues	35
V	Computed Cell Means Matrix of Body Weights (g) of Male Wistar Rats as a Function of Age (wks) and Activity Pattern	46
VI	Sheffé Multiple Comparisons of Activity Pattern Main Effects of Body Weights (g) in Young and Adult Male Rats	47
VII	Scheffé Multiple Comparisons of Age (wks) Main Effects of Body Weights (g) in Young and Adult Male Rats	47
VIII	Scheffé Multiple Comparisons of Age (wks) Main Effects for Total Collagen as Indicated by Hydroxyproline Concentrations (ug/mg stored weight) in Achilles Tendon of Young and Adult Male Rats	48

LIST OF TABLES (cont'd)

Table	Description	Page
IX	Scheffé Multiple Comparisons of Activity Pattern Main Effects for Total Collagen as Indicated by Hydroxyproline Concentrations (ug/mg stored weight) in Achilles Tendon of Young and Adult Male Rats	48
X	Scheffé Multiple Comparisons of Age (wks) Main Effects of Insoluble Fraction of Collagen in 0.045 M NaCl as Determined by Hydroxyproline Concentrations (ug/mg stored weight) in Achilles Tendon of Young and Adult Male Rats	49
XI	Scheffé Multiple Comparisons of Activity Pattern Main Effects of Insoluble Fraction of Collagen in 0.45 M NaCl as Determined by Hydroxyproline Concentrations (ug/mg stored weight) in Achilles Tendon of Young and Adult Male Rats	49

LIST OF FIGURES

Figure		Page
1	Endurance Continuous (EC) training scheme	13
2	Sprint Interval (SI) training scheme	15
3	Mean body weights (g) of sedentary controls (CS), endurance conditioned (EC), and sprint conditioned (SI) rats at 5, 15, 25, 32 weeks of age	19
4	Mean body weights (g) as a function of activity pattern (sedentary controls, CS; endurance continuous running, EC; and sprint-type interval running, SI of rats at 15, 25, 32 weeks of age	20
5	Levels of collagen structure	38

INTRODUCTION

Jogging has recently become a part of the lifestyle of many Canadians. The annual marathon hosted in Ottawa in May, 1978 included approximately 2000 participants ranging in ages from 9 to 60 years (1978 CBC news coverage). Not long ago such events were attended by a few select and elite individuals aspiring to set new records. It now appears that participation in running and other exercise activities holds increasing relevance especially for adults who do not intend to reach their physical potential. This fact stems from an awareness that certain physiological adaptations to submaximal types of conditioning programs have been shown to offset many deteriorative processes associated with aging (Astrand and Rodahl, 1977; DeVries, 1974; Bailey, 1973) and hence, enhance the quality of life (Wenger, 1977). Also, many older individuals who have remained physically active throughout life often appear 'younger' than their years. This has led to the assumption that a relationship exists between physical activity and an offsetting of the aging process (Allman, 1974; Malina, 1969).

The notion that biological aging is influenced by exercise, physical conditioning, or physical stress is not well substantiated by scientific inquiry since there is a paucity of studies in both animals and humans in which the factors of age and physical conditioning have been investigated for an extended period of time (Wilson, 1973; Malina, 1969). There are obvious difficulties when conducting longitudinal studies, particularly with humans.

Nevertheless, reported evidence (Astrand and Rodahl, 1977; Kash, 1976; Robinson et al, 1975; Kiessling, 1974) states that even into the fifth decade many of the functional parameters associated with strength and aerobic capacity can be maintained or, any decrease associated with age, minimized in response to vigorous physical training. Limitations of such findings may be due to the fact that the subjects studied represented the physiological elite (Wilson, 1973). Thus, it is recognized that the root cause of age-related effects at all higher, organizational levels of biological systems has a genetic basis (Hayflick, 1975, 1968). Therefore, it is by the use of laboratory animals of albino strain that much of the error variance due to individual differences can be diminished (Elias and Wright, 1974).

In addition to genetic determinants, developmental researchers maintain that adult capacity is, in part, a function of physical conditioning imposed during 'critical periods' of active growth and maturation (Bailey, 1973; Malina, 1969). This assumption underlies the principle of adaptation which states that the precise composition of a cell in terms of enzymatic and structural proteins can only be maintained by its particular pattern of functional activity (Perry, 1971 as quoted in Poortmans, 1975).

The tenet that structure subserves function is well demonstrated in connective tissue. All connective tissues (collagenous, elastic, mucous, reticular, osseus and cartilagenous) assume an architectural and mechanical function for the body (Dorland's Medical Dictionary,

1974). Yet each connective tissue has its own characteristic composition and structure suited to a specific function. Tendon, for example, forms a functional unit with muscle and bone and thus transmits forces generated by striated muscle across the articulations of joints in order that movement can occur (Hall, 1976; Verzar, 1963) or tension be produced. This particular activity pattern must be maintained within certain physiological tolerance limits since an excess of activity or immobilization and disuse is detrimental to the maintenance of functional connective tissue (Chvapil, 1973); shown by a decrease in structural stability and healing rate (Booth and Gould, 1975; Tipton and Matthes, 1975; Malina, 1969).

In 1945, Inglemark showed that conditioning young rabbits on a treadmill enlarged cross-sectional areas of achilles tendon and corresponding muscles compared to age-matched controls. In older animals studied, Inglemark noted hypertrophy of muscle, but not in the achilles tendon. Heikkinen and Vuori investigated the effect of 4 weeks of physical activity on the metabolism of connective tissue in aged mice (1972). The findings showed increased metabolism in achilles tendons of trained mice. However, Tipton, Martin (1975) and Viidik (1967) found no significant differences between trained and non-trained young adult rats when measuring the protein collagen dry weight (D.W.) of connective tissues.

In addition, Tipton and Martin (1975) noted that the physiochemical stability as an indicator of soluble and insoluble collagen

fractions in various solvents still remains to be studied in relation to training (Tipton and Martin, 1975). Obviously, more research is needed to answer the question of the effects of age and physical conditioning on connective tissues. Also, the intensity and duration of work is a consideration when studying physiological alterations due to training since it is well documented that adaptive responses tend to be specific (Astrand and Rodahl, 1977; Edington and Edgerton, 1976; DeVries, 1974). Byrd (1973) in his paper, "Effect of Controlled Mild Exercise on the Rate of Physiological Aging of Rats" stated the importance of clarifying the role of intensity of exercise with age since an optimal level exists, above or below which may hasten the aging process.

It appears that biological age need not be identical to chronological age (Ries, 1974; Chvapil, 1967; Verzar, 1963). The consistent reporting of step-wise changes of connective tissue concomitant with increasing age has made physiological age comparisons possible (Byrd, 1973; Hall, 1973; Heikkinen, 1972; Everitt, 1972; Chvapil, 1967 and 1973; Verzar, 1963, Sobel, 1962; Neuberger, 1953). A survey of the literature reveals that collagen, the primary structural protein of connective tissues responds to aging in the following ways:

1. Collagen will increase in concentration during growth and developmental phases of aging in order to meet the demands of the tissue's specialized function (Hall, 1976; Booth and Gould, 1975; Tipton and Martin, 1976).

2. Collagen exhibits an increase in content with increasing age that is similar between individuals and most species (Deyl et al, 1976; Viidik, 1973).

3. The binding forces that hold the collagen fibres together (known as cross-links) may increase in number (Verzar, 1963), change in character (Cannon and Davison, 1973), or both. Thus with age, collagen exhibits higher tensile strength, decreased visco-elasticity during mechanical testing (Booth and Gould, 1975; Tipton and Matthes et al, 1975; Viidik, 1967 and 1969) and increased physico-chemical stability as determined by the rate and degree of dissolution in various reagents (Mohan, 1976; Cannon and Davison, 1973; Verzar, 1963; Neuman and Logan, 1950).

4. Collagen fibres are largely acellular and alterations associated with aging or maturation can therefore be studied at the molecular level rather than at the tissue level (Cannon and Davison, 1973; Chvapil, 1967; Shock, 1966).

At the molecular level, all organisms have more in common that not (Prosser, 1950). The use of laboratory animals may provide a logical follow-up to an investigation of a relationship between prolonged physical conditioning and age on one major protein component of the body. Thus, the purpose of the study was to investigate the effects of age and sedentary, moderate (endurance) and intensive (sprint) physical

conditioning on body weights and chemical changes in collagen of achilles tendons during growth and maturation of rats.

DEFINITION OF TERMS

Age:

Chronological - expressed in terms of the period elapsed from the time of birth

Physiological or Biological
- expressed in terms of chronological age of a normal organism showing the same degree of anatomical and functional parameters of physiological development

Collagen:

- an albuminoid and member of the classification scleroproteins. Comprises 80% of the dried weight of tendons. Serves as the binding substance of skin, tendon, bone, cartilage and connective tissue generally. Recently, this protein complex has served as a biochemical determination of aging.

Conditioning:

- repeated bouts of exercise such that a physiological adaptative response is elicited

Development:

- process of growth and differentiation

Endurance conditioning:

- It is recognized that no one single energy source is recruited during physical conditioning, however, the primary energy source is aerobic.

Exercise:

- single bout or session of physical activity

Growth:

- normal process of an increase in size of an organism by accretion of tissues

Ponderal - pertains to weight and not necessarily size

DEFINITION OF TERMS (cont'd)

Hydroxyproline:

- imino acid, in nature 4-hydroxy-L-proline.
  It is found exclusively in collagen.
  Its measurement spectrophotometrically at 558 nm confers the biochemical measurement of collagen.

Maturation:

- process of becoming fully developed

Solubility characteristics of collagen:

- refers to physico-chemical stability
  With increasing age, collagen becomes less soluble in salt and weak acid solutions.

Sprint:

- It is recognized that no one single energy source is recruited during physical conditioning, however, the primary energy source encompassed by the term sprint is anaerobic.

Training:

- repeated bouts or sessions of exercise and involves a learning process

METHODS AND PROCEDURES

Experimental Design

In order to study the effects of age and prolonged physical conditioning on collagen in growing rats, the following independent variables were selected. Animals were studied at 15, 25 and 32 weeks of age (Factor A). Animals were exposed to either a sedentary control condition (CS), continuous endurance conditioning program (EC), or an interval sprint conditioning program (SI), (Factor B).

Three dependent variables were measured in order to investigate treatment effects.

1. *body weight (grams)*
2. *total hydroxyproline concentration (ug/mg stored tissue) in rat achilles tendon.*

   Hydroxyproline is virtually unique to collagen and therefore provides an 'in vivo' label for this protein (Tipton and Martin, 1975; Both and Gould, 1975; Bloom and Fawcett, 1975; Chvapil, 1973; Adams, 1970; Verzar, 1963; Woessner, 1961; Neuman and Logan, 1950).
3. *insoluble fraction of collagen in cold 0.45 M NaCl (ug/mg stored tissue).*

   It is generally accepted that salt extractable collagen is the most biologically active component of the collagen complex. The amount of collagen solubilized in weak salt solutions correlates inversely with increasing age (Weiss, 1967; Mohan, 1975; Booth and Gould, 1975; Cannon and Davison, 1973; Chvapil, 1973 and 1967; Shock, 1966; and Sjoerdsma, 1965).

Table I

Experimental Design, Experimental Unit Nomenclature and Sample Size for Age (Factor A) and Activity Pattern (Factor B)

FACTOR B

FACTOR A: CALENDAR AGE (WKS.)	ACTIVITY PATTERN: SEDENTARY CONTROL	ACTIVITY PATTERN: CONTINUOUS ENDURANCE	ACTIVITY PATTERN: INTERVAL SPRINT	ROW SUMS
15	CS-15 $n_{11} = 10$	EC-15 $n_{12} = 9$	SI-15 $n_{13} = 8$	$n_{1\cdot} = 27$
25	CS-25 $n_{21} = 11$	EC-25 $n_{22} = 7$	SI-25 $n_{23} = 9$	$n_{2\cdot} = 27$
32	CS-32 $n_{31} = 7$	EC-32 $n_{32} = 6$	SI-32 $n_{33} = 6$	$n_{3\cdot} = 19$
COLUMN SUMS	$n_{\cdot 1} = 28$	$n_{\cdot 2} = 22$	$n_{\cdot 3} = 23$	N=73

Therefore, the design was a 2-factor fixed effects model with 3 levels in each factor (Table 1).

Animal Care and Laboratory Conditions

Ninety male albino rats of Wistar strain at 4 - 5 weeks of age (80 - 100 g) were obtained from the director of the Health Science Animal Centre at the University of Alberta. The animals were housed individually in self-cleaning cages (45 x 69 cm), maintained on standard rat chow (Purina pellet form), and allowed water ad libitum. All animals were handled, cages were systematically rotated and soiled papers changed daily. Cages and water bottles were sterilized each week. Serial measurements of body weights were determined from the time the animals arrived in the laboratory until the prescribed day of sacrifice.

Since the rat is a nocturnal rodent and therefore most active at night (Prosser, 1950), it was necessary to reverse the day/night cycle (dark between 0600 and 1800) hours to facilitate conditioning.

Attrition

Due to injury, infection or a refusal to run, the attrition rate for this study was 18%.

Training Protocol

After an initial one week orientation to the laboratory setting, the EC and SI groups were familiarized with running on a Quinton Rodent

Treadmill (10 compartments of 9.5 x 45 cm, at a speed of 15 to 20 m/min. and 8% incline). Motivation to run was provided by an electric shock grid (50 v AC) at the back of each compartment.

The principle of progressive overload of duration and intensity (manipulated by time and/or treadmill speeds) was adhered to as shown in Figure 1 (Astrand and Rodahl, 1977; Edington and Edgerton, 1976; DeVries, 1974). The animals were trained twice a day, four days per week (Monday, Tuesday, Thursday, Friday). The days off were to permit recovery, enhance protein synthesis and to facilitate cleaning chores.

Continuous Endurance Conditioning

Stepwise increments of treadmill speed and duration of conditioning session (min) to a running speed of 30 m/min , 8% grade for a total of 60 minutes per day was achieved by the three endurance groups after 6 weeks (Figure 1). A maintenance program of a daily run of 60 minutes at 30 m/min was undertaken for an additional 4 weeks.

After the continuous endurance group were sacrificed at 15 weeks old (EC-15), EC-25 and EC-32 groups of animals were introduced to an increased treadmill speed of 35 m/min at training week 11. This intensity was further maintained for 60 minutes each day until EC-25 and EC-32 had completed 20 and 26 weeks of endurance-type activity respectively (Fig. 1).

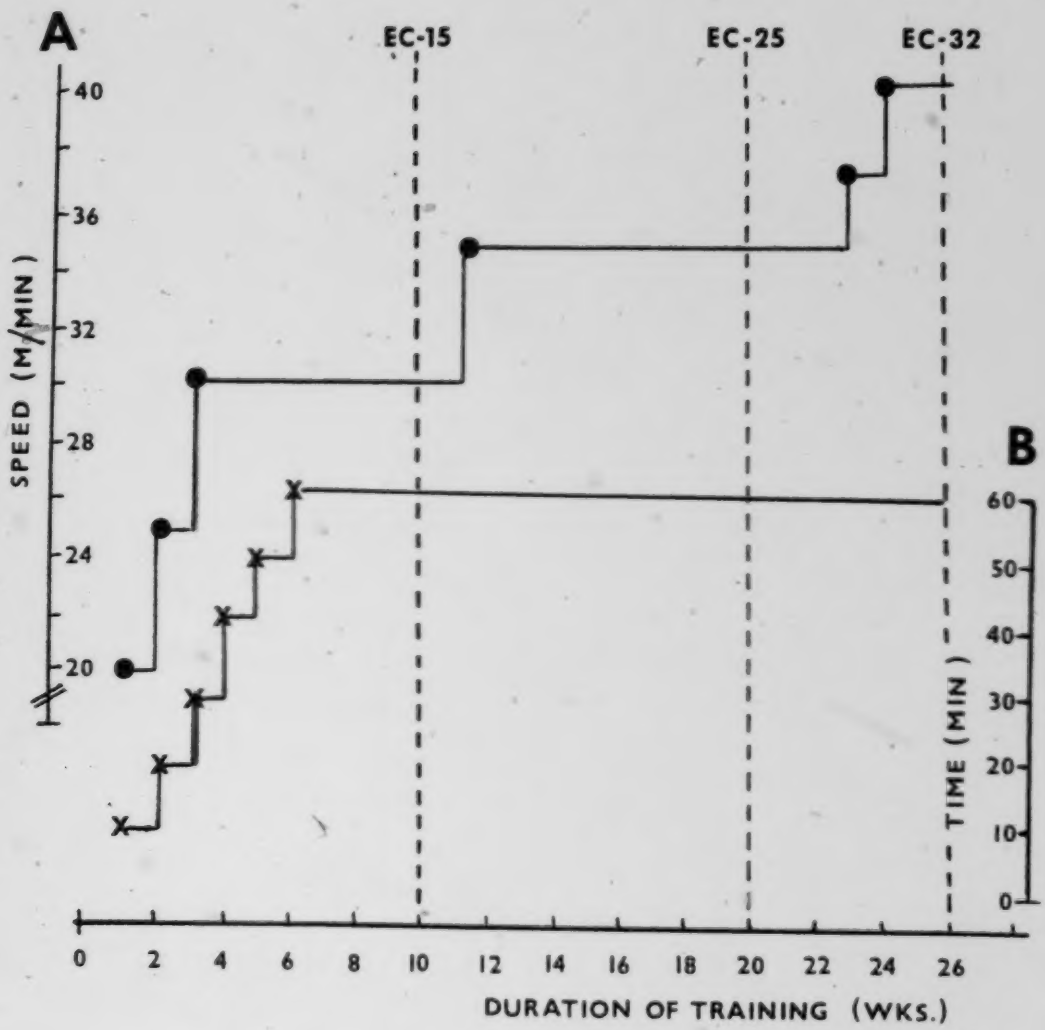


Figure 1: Endurance-Continuous (EC) Training Scheme

The upper curve (A) shows the running speed in metres per minute (m/min).
The lower curve (B) shows the time in minutes of each running period (4 times per week, twice each day).
EC sacrificed at 15, 25 and 32 weeks of age.

Interval Sprint Conditioning

All three levels of the SI group (SI-15, SI-25, SI-32) were run on the treadmill at a 30% grade for a maximum of 20 exercise bouts daily. The work/relief ratio was 15 seconds of running following by 20 seconds of rest (1:1.3).

From training weeks 1 through 6, running speeds were increased from 30 m/min to 60 m/min for the prescribed 20 bouts per day (Fig. 2). However, in order to acclimate the animals to sprinting at 80 m/min., the daily exercise bouts were reduced to 10 for the 7th week and then increased to 16 for the 8th training week (Fig. 2). The speed of 80 m/min. for 20 bouts per day was continued for an additional 9 weeks.

At approximately 23 weeks of age (18 weeks of sprint activity), the SI-25 and SI-32 groups were progressed to a treadmill speed of 85 m/min (Fig. 2).

SI-32 was further progressed to 20 bouts at 90 m/min. per day until sacrifice (Fig. 2).

Tissue Collection

Approximately 48 hours after the last exercise session, animals from the designated age groups were killed via decapitation with a hand operated small animal guillotine. Portions of the right and left achilles tendons of each rat were excised distal to the musculotendinous

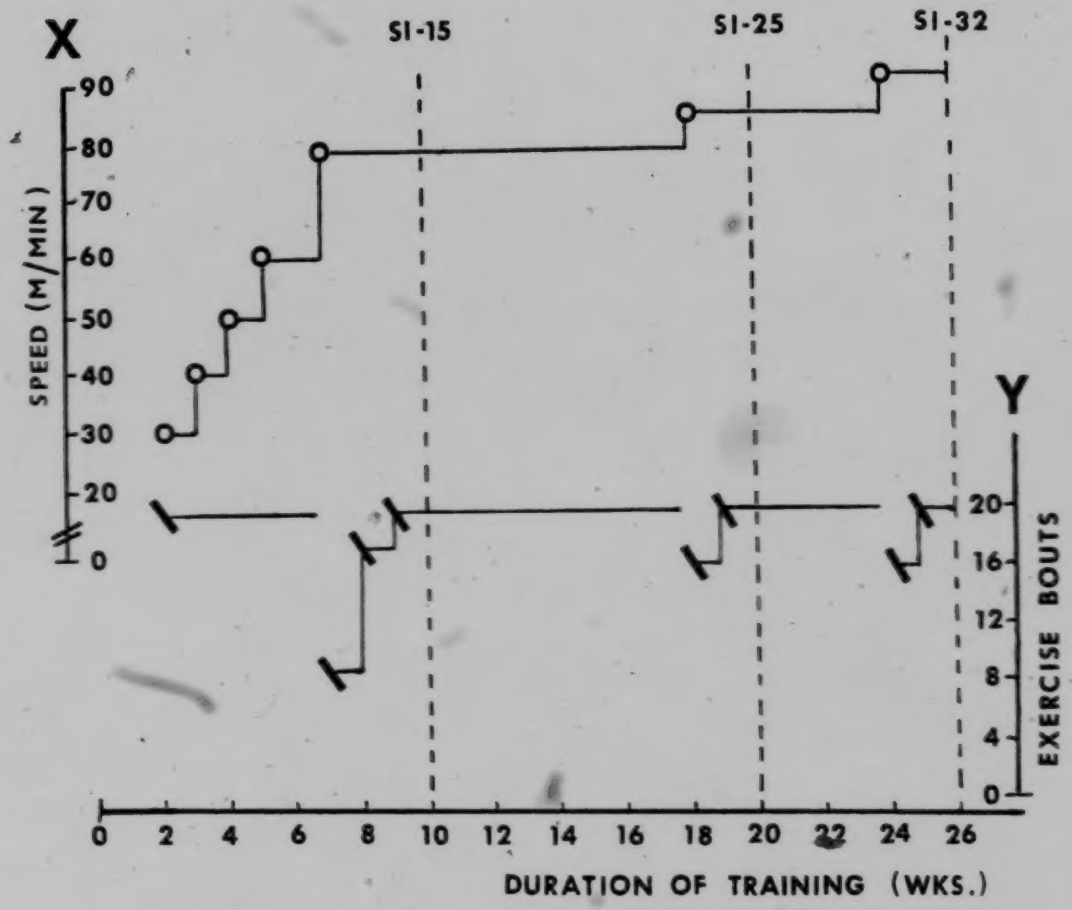


Figure 2: Sprint-Interval (SI) Training Scheme

Scale 'X' shows running speedo (m/min) performed during 26 weeks of conditioning.
Lower curve 'Y', illustrates the corresponding number of exercise bouts (15 sec run, 20 sec rest) performed.
SI sacrificed at 15, 25 and 32 weeks of age.

junction and proximal to its insertion onto the calcaneus. The tissue samples were then frozen in liquid nitrogen-cooled isopentane and stored at -80°C for subsequent analyses.

Tissue Analytical Techniques

Total hydroxyproline concentration (ug/mg stored tissue) of left achilles tendons was determined according to the method of Stegeman (1961) as modified by Bergman and Loxley (1963), (Appendix B).

The achilles tendons of the contralateral limb was assayed with a 0.45 M NaCl solution to yield an insoluble and soluble collagen fraction (Neuman and Logan, 1950). Subsequent determination for hydroxyproline concentration in the insoluble fraction followed Bergman and Loxley's (1963) colour reaction.

Tissue samples were not purified since virtually pure native collagen can be chemically obtained from unmodified achilles tendon (Tipton and Matthes, 1975; Cannon and Davison, 1973; Chvapil, 1967; Woessner, 1961).

Freezing Effects

Houck and Jacob (1961) reported that rat connective tissue lost about 30% wet weight due to freezing and storage at -15°C. After 21 days, tissue samples had only lost an additional 1% more weight. Hence, all tendon weights in this study are reported as stored tissue and corrected for weight.

Statistical Analysis

A 2-way analysis of variance (Ders: Anov 25, University of Alberta) was computed to reveal differences in:

1. *main effect of chronological age (wks).*
2. *main effect of activity pattern.*
3. *interaction effect between type of activity and age.*

Where F values were significant ($p < 0.05$) Scheffé multiple contrasts for simple main effects were calculated for body weights (grams), total hydroxyproline concentration (ug/mg stored tissue), and insoluble fraction in 0.45 M NaCl (hydroxyproline concentration, ng/mg stored weight).

RESULTS

Initial body weights (grams) for the different groups did not differ ($p < 0.05$). However, with age, a significant increase in body weight occurred between 15 week old animals compared to both 25 and 32 week groups (Fig. 3). According to the data (Appendix C), aging after 25 weeks in the rat does not produce an increase in ponderal growth (Fig. 3). In terms of activity pattern, significantly lower body weights were found for endurance conditioned rats compared to sedentary controls. Apparently, the sprint conditioning stimulus did not elicit changes in body weights with respect to either sedentary or endurance conditioned animals (Fig. 4).

The results also showed that total collagen of rat achilles tendon increased significantly with aging between 15 and 32 weeks (Table II). It was also observed that 10 weeks of aging (15 to 25 weeks) did not affect ($p < 0.05$) collagen content of rat achilles tendon. In addition, endurance or sprint conditioning did not induce significant changes in collagen content in achilles tendon compared to sedentary animals (Table II).

No statistical difference existed in insoluble fractions across age or between conditioning groups ($p < 0.05$) (Fig. 5).

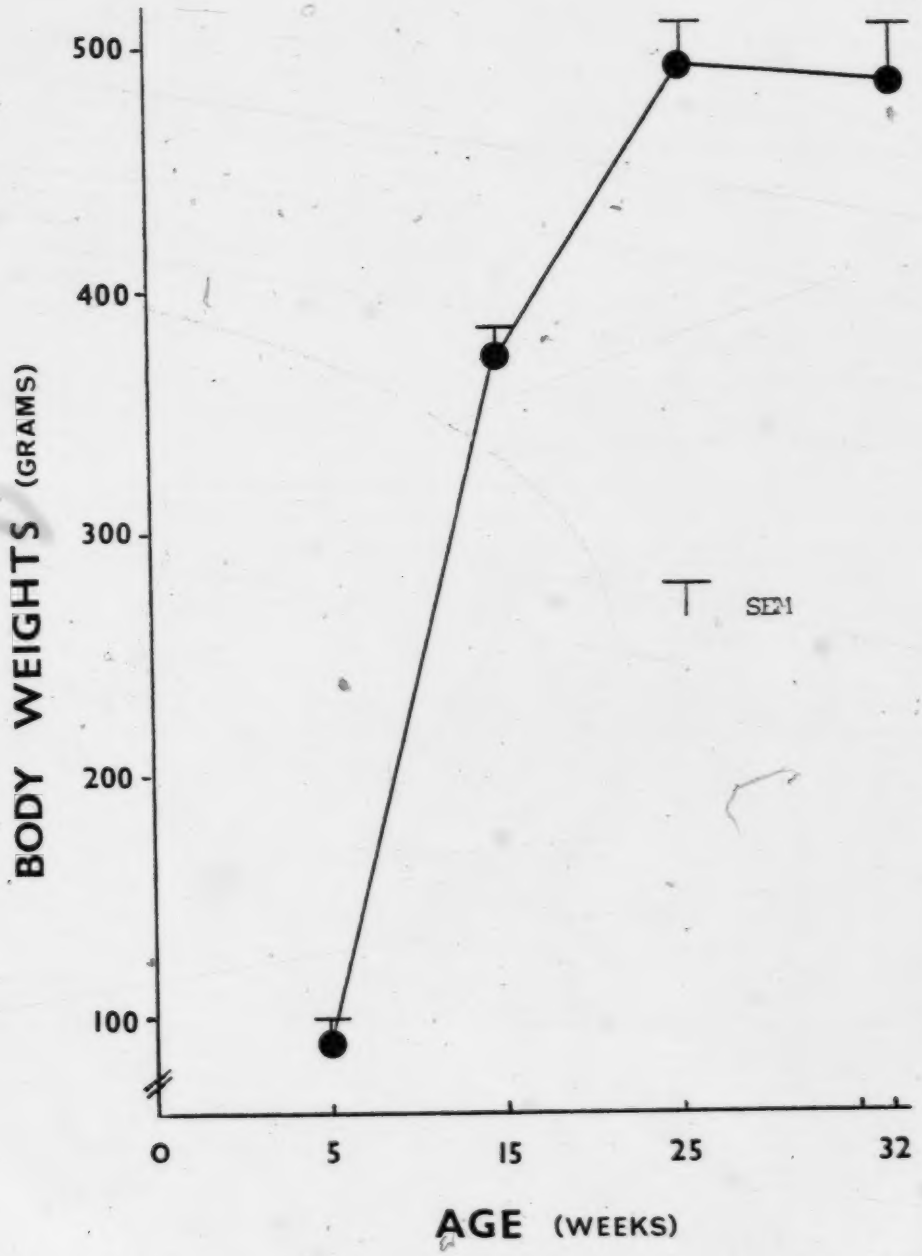


Figure 3: Mean body weights (grams) of sedentary controls (CS), endurance conditioned (EC) and sprint conditioned (SI) rats at 5, 15, 25 and 32 weeks of age.

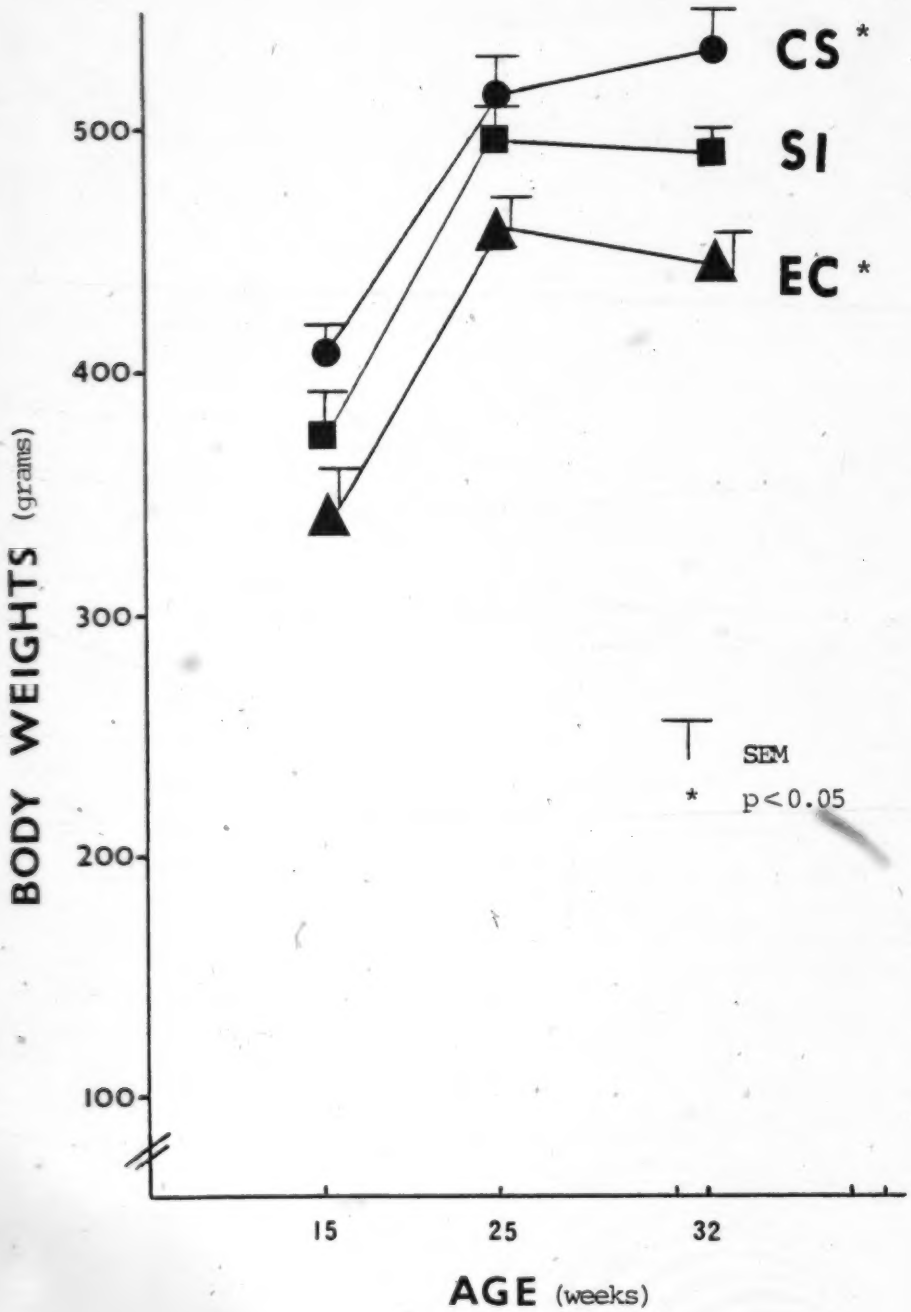


Figure 4: Mean body weights (grams) as a function of activity pattern (sedentary controls CS, endurance/continuous running EC, and sprint-type running/interval SI) of rats at 15, 25, 32 weeks of age.

DISCUSSION

Body weights and collagenous modifications (total collagen content as shown by hydroxyproline concentration and insolubility fraction of collagen in 0.45 M NaCl) in achilles tendon have been studied in three age groups of rats (15, 25, 32 weeks) adapted in the course of 26 weeks to moderate and intensive physical conditioning. The results of this study substantiate previous findings on body weights of rodents where ponderal growth progresses through an early exponential phase followed by a slow asymptotic phase at maturity (Borer and Kaplan, 1977). Specifically, 15 week old rats gained 100 ± 5 grams by the 25th week while no increase in body weight was noted by 32 weeks of age. This evidence confirms the premise that chronological ages of 15, 25 and 32 weeks in the laboratory rats studied included both growth and maturational phases of the aging process.

There was no differential effect on body weight brought about by endurance and sprint conditioned groups. However, as demonstrated in previous studies (Wilkinson, 1977 and Borer, 1977) endurance trained animals showed a reduced body weight gain (p 0.05) when compared to sprint and sedentary groups. This lower body weight was probably due to increased energy expenditure, decreased food intake, or both (Wilkinson et al., 1977). Sprint conditioning did not result in significantly different body weights in comparison to endurance trained and non-conditioned animals which suggests that caloric intake and expenditure were similar to both EC and CS groups.

The data summarized in Table II demonstrates that aging from 15 to 32 weeks induced a significant increase in collagen content in the achilles tendon as indicated by an increase in hydroxyproline concentration in CS, EC and SI groups. This is in agreement with Mohan (1975), and Sobel (1962) who stated that collagen of mammals accumulated rapidly during growth. An investigation of skin and tendon from 2 and 80 year human necropsies also showed an increase in total hydroxyproline concentration (Deyl et al., 1971). However, the age-related progressive increase of collagen found (Table II) is apparently unique to the rat since most other species accumulate collagen at a considerably slower rate after skeletal and sexual maturity (Viidik, 1973).

Results of studies investigating collagen content as measured biochemically by determining hydroxyproline concentration do not agree that this parameter increases after physical conditioning. Kiiskinen and Heikkinen (1973) trained 2 week old mice for 3 to 22 weeks and found an increased hydroxyproline content in tendons compared to non-trained controls. Heikkinen and Vuori (1972) also found exercise-related effects of collagen content in achilles tendons of old mice (12 - 14 months) trained for only four weeks. Viidik (1967), Tipton and Martin (1975) conditioned young rabbits and rats for 40 weeks and 15 weeks respectively. No alterations of collagen content due to exercise were found.

TABLE II

COMPUTED CELL MEANS MATRIX OF TOTAL COLLAGEN AS INDICATED BY HYDROXYPROLINE CONCENTRATIONS (ug/mg) OF ACHILLES TENDON AS A FUNCTION OF AGE (WKS) AND ACTIVITY PATTERN IN YOUNG AND ADULT MALE RATS

FACTOR A AGE (WKS)		FACTOR B ACTIVITY PATTERN		
		Control Sedentary CS	Endurance Continuous EC	Sprint Interval SI
	15	34.70 ± 6.99 $n_{11}=9$	60.38 ± 12.15 $n_{12}=8$	40.50 ± 7.1 $n_{13}=7$
	25	55.30 ± 10.44 $n_{21}=7$	76.91 ± 13.2 $n_{22}=7$	74.42 ± 23.0 $n_{23}=6$
	32	99.64 ± 33.7 $n_{31}=7$	83.60 ± 22.90 $n_{32}=6$	82.06 ± 30.1 $n_{33}=4$

In the present study, moderate and intensive physical conditioning programs applied over 26 weeks also indicated that, to date, no convincing evidence exists to suggest that training affects collagen content of achilles tendon in the rat.

Hypotheses from these findings can be made although more work would be needed for verification. Just as a certain minimum threshold of cardiovascular response (heart rate) must be met in order for improved aerobic capacity (Astrand and Rodahl, 1977) the possibility exists that the intensity or duration of physical conditioning must satisfy a certain specific level in order to induce structural adaptations at the molecular level. Certainly, procedural defects in biochemical analysis could reveal differences due to training where none exists. However, it is more likely that discrepancies of collagen alterations due to exercise may, in fact, be a function of the species of laboratory animal studied. The rat, for example, retains some potential for skeletal growth throughout life, while epiphyseal growth centres close in carnivores and primates (Borer, 1977 and Bourliere, 1954). More research investigating the relationship of physical conditioning and collagenous modifications using different animal models is needed.

The physico-chemical stability of collagen, in terms of its solubility characteristics has been studied in relation to both biological and chronological aging (Weiss, 1977; Hall, 1976; Mohan, 1975; Booth and Gould, 1975; Deyl, et al., 1973; Cannon and Davison, 1973; Chvapil, 1973, 1967; Everitt, 1972; Adams, 1970;

Shock, 1966; Sjoerdsma, 1965). Connective tissues (skin, bone, tendon, ligament, cartilage) of several species studied showed that progressively less collagen can be extracted by saline and acid reagents from older tissues (Cannon and Davison, 1973). However, Chvapil (1973), studying the growth pattern of collagen (lung, heart and femur) of 7.5 and 32 week old rats found that compared to domesticated animals of equal age, the differential effect on collagen solubility was attributed to an offsetting of the aging process in wild rats. Whether physical conditioning has any influence on soluble and insoluble fractions of collagen is not documented elsewhere (Tipton and Martin, 1975). The findings of this study show that the effects of age, sedentary, moderate and intensive physical conditioning do not modify the insoluble fraction of a 0.45 M NaCl extraction of achilles tendon in rats. A survey of the data in Table III reveals that variability of hydroxyproline concentrations was too great to permit statistically significant differences. However, insolubility fractions, as measured by hydroxyproline concentrations have been reported (Table III) in order that the findings will not necessarily be deemed unimportant as significance testing may dictate.

The limitations of this inquiry are recognized. Certainly, the changes described in achilles tendon of young and mature rats represented only a fraction of the total dynamic processes of the entire body (Chvapil, 1967). Furthermore, the use of laboratory animals has obvious limitations when generalizing to the human population.

TABLE III

COMPUTED CELL MEANS MATRIX OF
INSOLUBLE FRACTION OF COLLAGEN AS INDICATED BY
HYDROXYPROLINE CONCENTRATIONS (ug/mg) IN 0.45 M NaCl
FROM ACHILLES TENDON AS A FUNCTION OF AGE (WKS)
AND ACTIVITY PATTERN IN YOUNG AND ADULT MALE RATS

		FACTOR B ACTIVITY PATTERN		
		Control Sedentary CS	Endurance Continuous EC	Sprint Interval SI
FACTOR A AGE (WKS)	15	48.38 ± 16.3 $n_{11}=9$	79.84 ± 21.5 $n_{12}=7$	61.42 ± 18.3 $n_{13}=5$
	25	69.38 ± 18.7 $n_{21}=9$	75.48 ± 26.3 $n_{22}=6$	65.37 ± 22.7 $n_{23}=7$
	32	76.10 ± 17.0 $n_{31}=7$	131.24 ± 13.9 $n_{32}=4$	69.46 ± 25.0 $n_{33}=8$

It is concluded that:

1. Body weights of endurance conditioned rats were decreased compared to sedentary free eating controls.
2. Body weights of 15 week old rats significantly increased by 25 weeks; however, weight gain between 25 and 32 weeks of age was not demonstrated.
3. Total collagen content of achilles tendon increased as a function of age between 15 and 32 weeks.
4. Moderate and intensive physical conditioning of an endurance and sprint nature did not affect total collagen content of achilles tendon.
5. The fraction of insoluble collagen in a 0.45 M NaCl solution was not affected by age, sedentary, endurance or sprint conditioning treatments.

Recommendations

The present study suggests that no conclusive evidence exists to substantiate the notion that there is a relationship between physical conditioning and aging in collagen of achilles tendons of 15, 25, and 32 week old rats.

It is therefore recommended that training and aging studies investigate alterations in collagen in prepubertal and senile age ranges and different laboratory animal models.

REFERENCES

ADAMS, E. Metabolism of proline and hydroxyproline. In, International Review of Connective Tissue Research. Ed. by D. A. Hall and D. S. Jackson. Vol. 5, 1970.

ALLMAN, F. L. Jr. Premature physical deterioration: A reversible disease. J. of Sports Med. 2 (5) : 295-296, 1974.

ASTRAND, P.-O. and RODAHL, K. Textbook of Work Physiology, 2nd edition. McGraw-Hill Book Co., New York, Toronto. 1977.

BAILEY, D. A. Exercise, fitness and physical education for the growing child - A concern. Can. J. of Public Health. 64 : 421-430, 1973.

BERGMAN, M. and LOXLEY, R. Two improved and simplified methods for the spectrophotometric determination of collagen. Anal. Chem. 35 : 1,961 - 1,965, 1963.

BLOOM, Wm. and FAWCETT, D. W. A Textbook of Histology, W. B. Saunders Co., Philadelphia, 1975.

BOHINSKI, Robert C. Modern Concepts in Biochemistry, 2nd edition. Allyn and Bacon, Inc. London, Toronto, 1976.

BOOTH, F. and GOULD, E. W. Effects of training and disuse on connective tissues. In, Exercise and Sport Sciences Riviews. Ed. by J. H. Willmore and J. F. Keogh. 3 : 83 - 111, 1975.

BORER, K. T. and KAPLAN, L. R. Exercise-induced growth in golden hamsters: Effects of age, weight, and activity level. Physiol. and Behaviour. 18 : 29-34, 1977.

BOURLIERE, F. Role of comparative physiology in studies of aging. In, Problems of Aging. Ed. by N. W. Shock, Josiah Macy, Jr. Foundation Progress Associates, Caldwell, N. J., 1954.

BYRD, R. J. The effect of controlled, mild exercise on the rate of physiological aging of rats. The J. of Sports Med. and Physical Fitness. 13 (1) : 1-3, 1973.

CANNON, D. J. and DAVISON, D.F. Cross-linking and aging in rat tendon collagen. Exp. Geront. 8 : 51-62, 1973.

CHVAPIL, Milos. Physiology of Connective Tissue. Czechoslovak Medical Press, Butterworth and Co. Ltd. Prague, 1967.

CHVAPIL, M., BARTOS, D. and BARTOS, F. Effect of long-term physical stress on collagen growth in the lung, heart and femur of young and adult rats. Gerontologia. 19 : pp.263-270, 1973.

CHVAPIL, M., KROENTHAL, R. L. and VAN WINKLE, Jr., W. Medical and surgical applications of collagen. In, International Review of Connective Tissue Research. Ed. by D.A. Hall and D. S. Jackson. Vol. 6, 1973.

DeVRIES, H. A. Physiology of Exercise. Wm. C. Brown Co., Iowa, 1974.

DEYL, Z., JURICOVA, M. et al. Aging of the connective tissue: collagen cross-linking in animals of different species and equal age. Exp. Geront. 6 : 227-233, 1971.

DONALDSON, H. H. and CONROW, S. B. Quantitive studies on the growth of the skeleton of the albino rat. Am. J. Anat. 26 : 237-314, 1919.

DORLAND'S ILLUSTRATED MEDICAL DICTIONARY 25th edition. W. B. Saunders Co. Philadelphia, London, Toronto. 1974.

EASTOE, J. E. and COURTS, A. Practical Analytical Methods for Connective Tissue Proteins. E. and F. N. Spon Ltd., London.

EDINGTON, D. W. and EDGERTON, V. R. The Biology of Physical Activity. Houghton Mifflin Co., Boston. 1976.

ELLIOTT, G. M. The effects of exercise on structural growth. J. C.A.H.P.E.R. 36:21, 1970.

EVERITT, A. V. and DELBRIDGE, L. Two phases of collagen aging in the tail tendon of hypophysectomized rats. Exp. Geront. 7 : 45-51, 1972.

FARRIS, E. J. and GRIFFITH, J. Q. (editors). The Rat in Laboratory Investigation. J. B. Lippincott Co., Philadelphia, Montreal. 1949.

FOX, E. L. and MATHEWS, D.K. Interval Training. W. B. Saunders Co., Philadelphia, London, Toronto, 1974.

FUJII, K.; KUBOKI, Y. and SASAKI, S. Aging of human bone and articular cartilage collagen. Geront. 22 : 363-370, 1976.

GIONET, N. The Effects of Growth, Araerobic and Aerobic Training on Protein Nucleic Acids and Connective Tissue in the left ventrical of Male Rats. A PhD thesis University of Alberta, Edmonton, Alberta, 1977.

HALL, D. A. The Aging of Connective Tissue. Academic Press; London, New York, San Francisco, 1976.

HAYFLICK, L. Current theories of biological aging. Federation Proceedings. 34 : 9-13, 1975.

HAYFLICK, L. Human cells and aging. In, Organic Chemistry of Life. W. H. Freeman and Co., San Francisco. 1968.

HEIKKINEN, E. and VUORI, I. Effect of physical activity on the metabolism of collagen in aged mice. Acta. Physiol. Scand. 84 : 543-549, 1972.

HOUK, J. C. and JACOB, R. A. The chemistry of local dermal inflammation. J. Invest Derm. 36 : 451-456, 1961.

JACKSON, S.F.: HARKNESS, R. D.: PARTRIDGE, S. M. and TRISTRAM, G. F. Structure and Function of Connective and Skeletal Tissue, 1965.

KARLSON, P. Introduction to Modern Biochemistry, 2nd edition. Academic Press, New York, London. 1965.

KASCH, F. W. and WALLACE, J. P. Physiological variables during 10 years of endurance exercise. Med. and Sci. in Sports. 8 (1) : 5-8, 1976

KIISKINEN, A. Physical training and connective tissues in young mice. Physical properties of achilles tendons and long bones. Growth. 41 (2) : 123-137, 1977.

KIISKINEN, A. and HEIKKINEN, E. Effect of prolonged physical training on the development of connective tissues in growing mice. In, Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. R. Poortmans. Birkhause Verlag Basel. pp. 253-261, 1975.

MALINA, R. M. Exercise as an influence upon growth. Clin. Pediatr. 8 : 16, 1969.

McGILVERY, R. W. Biochemistry - a functional approach. W. B. Saunders Co., Philadelphia, London, Toronto, 1970.

MILLER, E. J. and MATUKAS, V. J. Biosynthesis of collagen. Federation Proceedings. 33 (5) : 1,197 - 1,218, 1974.

MOHAN, S. and RADHA, E. Collagen in aging muscles. Experimentia 31 (10) : 1,181-1,183, 1975.

NEUBERGER, A. and SLACK, H. G. B. The metabolism of collagen from liver, bone, skin and tendon in the normal rat. Biochem. J. 53 : 47-52, 1953.

NEUMAN, R. E. and LOGAN, M. A. The determination of hydroxyproline. J. Biol. Chem. 184 : 299. 1950.

PLANT, S. M. Animal models in developmental research. Pediatric Clinics of North America. 619-631, 1975. 22 (3) : 619-631, 1975.

POORTMANS, J. R. Effects of long lasting physical exercise and training on protein metabolism. In, Metabolic Adaptation to Prolonged Physical Exercise. Edited by H. Howald and J. R. Poortmans. Birkhauser Verlag Basel, pp. 212-228, 1975.

PROCKOP, D. J. A specific method for the analysis of hydroxyproline in tissues and urine. Analyt. Biochem. 1 : 228-239, 1960.

PROSSER, C. L. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia. 1950.

REARDON, F. D. The effect of glucose supplement on glycogen storage, utilization, and performance capacity during high intensity work. A PhD Thesis. Department of Phys. Ed. University of Alberta, Edmonton. 1975.

RIES, W. Problems associated with biological age. Exp. Geront. 9 : 145-149, 1974.

ROSMUS, J.; VANČIKOVA, O. and DEYL, Z. Modelling of cross-linking interactions in the terminal region of the collagen molecule. Exp. Geront. 8 : 39-43, 1973.

RUBIN, A. L.; DRAKE, M.P.; DAVISON, P. F.; PFAHL, D.; SPEAKMAN, P. T. and SCHMITT, F. O. Effects of pepsin treatment on the interaction properties of tropocollagen molecules. Biochemistry. 4 (2) : 181-190, 1975.

SHEPHERD, R. E. and GOLLNICK, P. D. Oxygen uptake of rats at different work intensities. Pflugers Arch. 362 : 219-222, 1976.

SHOCK, N. W. Perspectives in Experimental Gerontology. Charles C. Thomas, Springfield, Illinois. 1966.

SHOCK, N. W. Physical activity and the rate of aging. Canada. Med. Ass. J. 96 : 836-840, 1967.

SJOERDSMA, A. S. Hydroxyproline and collagen metabolism. Ann. In. Med. 63 : 672-674. 1965.

SOBEL, H. Role of connective tissue in gerontology. In, Medical and Clinical Aspects of Aging. Edited by H. T. Blumenthal. Columbia University Press. New York, London. 1962.

STRYER, L. Biochemistry. W. H. Freeman and Co. San Francisco. 1975.

TIPTON, C. M.; MATTHES, R. D.; MAYNARD, J. A. and CAREY, R. A. The influence of physical activity on ligaments and tendons. Med. and Sci. in Sports. 7 (3) : 165-175, 1975.

TIPTON, C. M.; MARTIN, R. K.; MATTHES, R. D. and CAREY, R. A. Hydroxyproline concentrations in ligaments from trained and non-trained rats. In, Metabolic Adaptation to Prolonged Physical Exercise. Birkhauser Verlag Basel. pp. 262-267, 1975.

VERZAR, F. The aging of collagen. Sci. Amer. 208 (4) : 104-114. 1963.

VIIDIK, A. Functional properties of collagenous tissues. In, International Review of Connective Tissue Research. Edited by D. A. Hall and D. S. Jackson. Academic Press, New York, London. pp. 127-215, 1973.

VIIDIK, A. Tensile strength properties of achilles tendon systems in trained and untrained rabbits. Acta. Orthop. Scandinav. 40 : 261-272, 1969.

VIIDIK, A. The effect of training on the tensile strength of isolated rabbit tendons. Scand. J. Plast. Reconstr. Surg. 1 : 141-147, 1967.

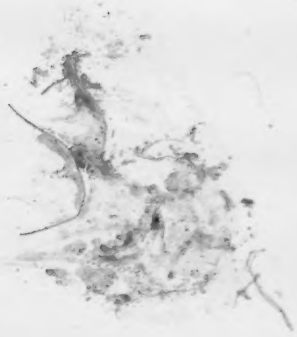
WEISS, J. B. Enzymatic degradation of collagen. In, International Review of Connective Tissue Research. Vol. 7, 1967.

WENGER, H. A. Alterations in physical work capacity with age. Unpublished paper. Address given to the city of Edmonton Police Department.

WILKINSON, J. G.; JOBIN, J.; REARDON, F.D.; MACNAB, R. B. J.; WENGER, H. A. Aerobic and anaerobic training: effects on aging patterns in rat skeletal muscle fibre types. Unpublished paper. University of Alberta, Edmonton, 1977.

WILSON, P. D. Enzyme changes in aging mammals. Gerontol. 19 : 79-125, 1973.

WOESSNER, J. F. Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Archives of Biochemistry and Biophysics. 93 : 440-447, 1961.



APPENDIX A

REVIEW OF LITERATURE

The reactivity of connective tissues, specifically achilles tendon, has been examined in relation to the factors of age and physical conditioning. Some excellent reviews and studies pertaining to the influence of aging and exercise on collagen have been mentioned (Introduction), (Hall, 1976, Edington and Edgerton, 1976; Booth and Gould, 1975; Tipton and Martin, 1975; Tipton and Matthes, 1975; Viidik, 1973; Chvapil, 1973 and 1967; Everitt, 1972; Heikkinen and Vouri, 1972; Verzar, 1963). It is evident that there is accumulating data concerning collagen changes with aging and training which may often conflict or present a confusing picture. Thus, Table IV was compiled in order to facilitate interpretation and comparisons of findings.

It is not enough to understand endogenous and exogenous effects on collagen without also knowledge of structure and function. Chvapil's Physiology of Connective Tissue (1967) presents an especially extensive and dynamic account of this tissue formerly thought to be inert (Tipton, 1975). Certainly, connective tissue researchers such as Chvapil, 1973; Verzar, 1963; Jackson, 1965; Hall, 1976; Bohinski, 1976; and Stryer, 1975 provided a more complex and detailed treatment, and the reader is referred to their work. However, a modest account of the physiology and structure of collagen has been made in order to provide a foundation for an elucidation of factors investigated (age, physical conditioning) in the present study.

TABLE IV

CURRENT STUDIES INVESTIGATING THE EFFECTS OF PHYSICAL CONDITIONING AND/OR AGE ON COLLAGEN CONTENT AS DETERMINED BY HYDROXYPROLINE ANALYSES OF CONNECTIVE TISSUES

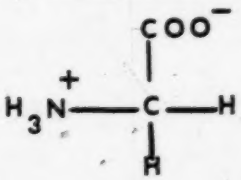
INVESTIGATOR	YEAR	ANIMAL	TISSUE	AGE (WKS)	TOTAL COLLAGEN CONTROL	TRAINED	MODE OF CONDITIONING (WKS)	DURATION	PROBABILITY (SIGNIFICANCE)
Sinclair	1978	Rat	Achilles	15	35.0	51.0	Endurance 35 m/min/60 min	10-26	N.S.
		Wistar	Tendon	25	55.0	75.0	Sprint		
				32	99.0	82.5	85 m/min/20 bouts		
					units: ug/mg stored weight				
Gionet	1977	Rat	heart	4-5	0.31	Anaerobic Aerobic	Endurance	10-26	
		Wistar		14-15	0.47	0.46 0.42	35 m/min/60 min		
				25	0.46	0.49 0.48	Sprint		
				32-33	0.63	0.62 0.58	85 m/min/20 bouts		0.05
Kiiskinen & Heikkinen	1976	Mice Albino	skin	2	52.22	70.16		3-22	0.05
				24	78.04	103.19	18m/min for 1 1/2 hr.		0.001
					units: ug/mg				
Kiiskinen & Heikkinen	1976	Mice Albino	heart	3	3.38	3.37	Moderate	22	N.S.
				12	3.64	3.84			
				22	4.82	5.57			
Tipton, Martin et al	1975	Rats Sprague Dawley	liga-ments	8	75.0	75.0	Sprint	10-15	N.S.
				12	86.0	78.0	Endurance		
					units: ug/mg D.W.				
Kiiskinen & Heikkinen	1975	Mice Albino	Achilles tendon	12	115.27	116.59	Light	10-15	N.S.
				17	124.53	134.45	Heavy		
					units: ug/mg				
Chvapil	1973	Rats Sprague	Femur	7-30	21.1	21.8	Moderate 24 m/min	7	N.S.
					units: mg/g				
Heikkinen & Vuori	1972	Mice Albino	Achilles tendon	12-14	0.54	0.63	Moderate	4	0.02
					units: Hydroxyproline / Nitrogen				

The word collagen comes from the Greek word, 'to produce glue'. In every day living, it is seen as the jelly substance of cooked ham or boiled chicken. This event, as well as the shrinking of tendon upon heating prompted Verzar in the early 1950's to study collagen as a possible index of biological aging (Verzar, 1963).

Collagen distribution in the body testifies to its biological importance since it comprises about 1/3 of the total body protein (Bohinski, 1976; Stryer, 1975). Furthermore, Sobel (1962) stated that 'all the cells of the organism except those floating free in the blood stream are dependent upon connective tissues for contact with the external environment'. A member of the classification scleroproteins (Karlson, 1965), collagen serves as the binding substance of skin, muscular and vascular systems (Dorland's Medical Dictionary, 1974).

The collagen complex can be more readily conceptualized when considering a rope. There are three main strands or peptide chains comprising the rope known as tropocollagen. These strands are twisted in a right-handed helix and in turn each one of the three peptide chains is individually coiled in a left-handed helix, thus, rendering the collagenous tissue 'flexible' while offering resistance to longitudinal forces (Hall, 1976).

The characteristic helical rod conformation described is of a defined 3000 Å in length and 15 Å across. This is, in part, due to its amino acid composition and sequence. Apparently, a regular amino acid order has not been identified (Stryer, 1975). However, it is well established that almost every 1/3 residue is glycine (Bohinski, 1976; Bloom and Fawcett, 1975; McGilvery, 1970; Karlson, 1965). This fact can be explained in terms of the molecular structure of glycine. As a relatively 'compact' molecule (Stryer, 1975), glycine easily occupies the interior position of the helix while the imino



acids (secondary amino acids) of proline and hydroxyproline can be more readily accommodated on the outer helix of the collagen complex (Stryer, 1975), (Fig. 5).

Hydroxyproline (in nature, 4-hydroxy-L-proline) has been used as the 'defining' component for collagen (Hall, 1976; Bergman and Loxley, 1963; Woessner, 1961). It is synthesized by hydroxylation of proline residues and not as a free amino acid (Adams, 1970) and its accumulation is an indicator of steric rigidity of the collagen complex (Chvapil, 1967). The rigidity of the complex comes about by cross-linkages inter- and intramolecularly (Bohinski, 1976). These cross-links vary in binding capacity depending on the electric charge of the elements (for example, hydrogen bonding vs. covalent bonding). The hydroxyproline complexes liberated from collagen due to acidic and extreme heat conditions (130°C) as performed in this study, was measured spectrophotometrically at 558 nm (Bergman and Loxley, 1963) thus conferring the biochemical measurement of collagen.

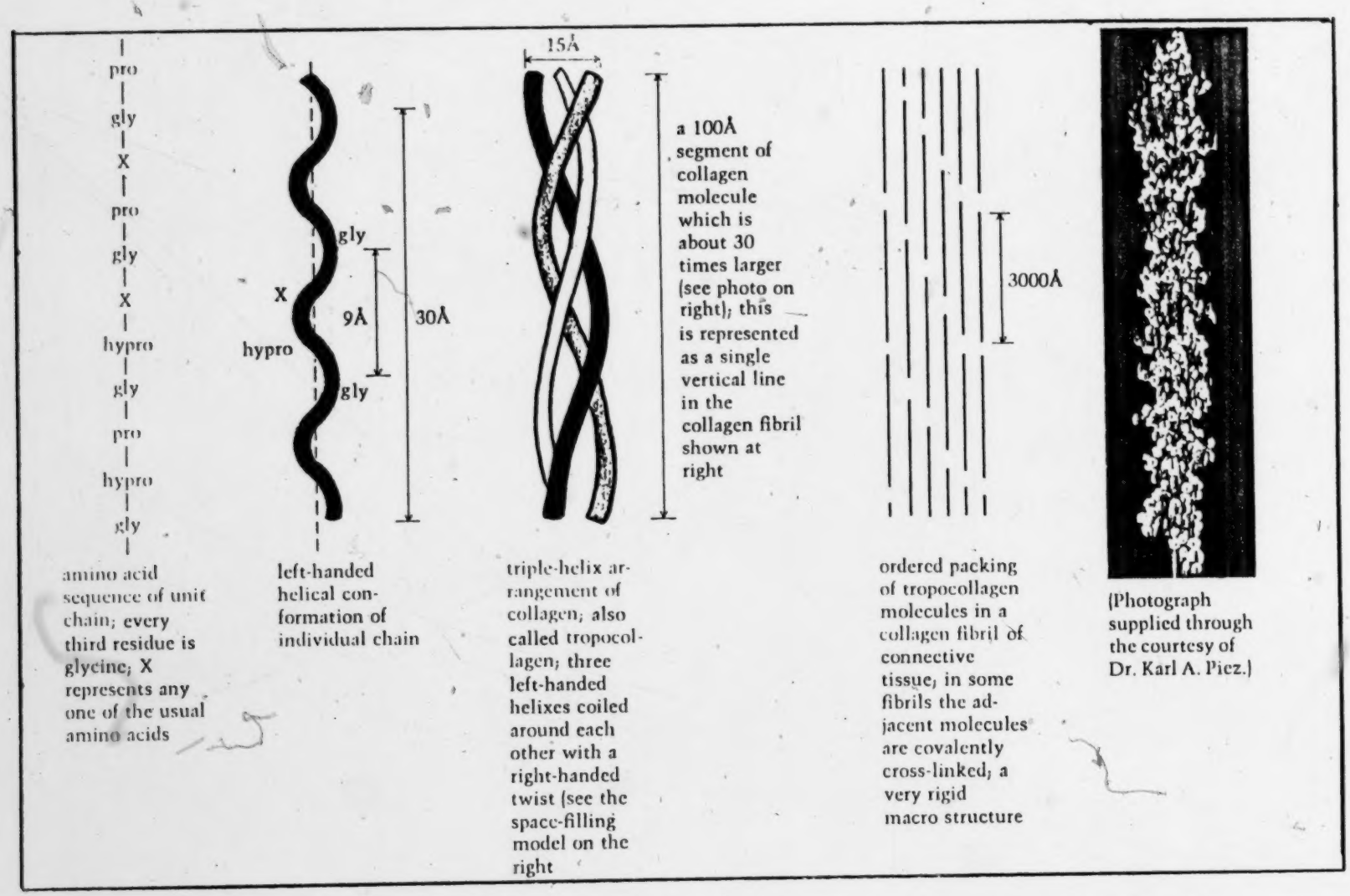

pro
gly
X
pro
gly
X
hypro
gly
pro
hypro
gly
amino acid sequence of unit chain; every third residue is glycine; X represents any one of the usual amino acids
gly
X
hypro
gly
9Å
30Å
left-handed helical conformation of individual chain
15Å
a 100Å segment of collagen molecule which is about 30 times larger (see photo on right); this is represented as a single vertical line in the collagen fibril shown at right
triple-helix arrangement of collagen; also called tropocollagen; three left-handed helixes coiled around each other with a right-handed twist (see the space-filling model on the right
3000Å
ordered packing of tropocollagen molecules in a collagen fibril of connective tissue; in some fibrils the adjacent molecules are covalently cross-linked; a very rigid macro structure
(Photograph supplied through the courtesy of Dr. Karl A. Piez.)

Figure 5: Levels of collagen structure. (Taken from R.C. Bohinski's Modern Concepts in Biochemistry, 1976).

APPENDIX B

SPECTROPHOTOMETRIC ESTIMATION OF HYDROXYPROLINE

REAGENTS: as described by Bergman and Loxley, 1963.

1. Oxidant solution

   a. A 7% w/v aqueous solution of Chloramine T (sodium salt of N-chloro-p-toluenesulfonamide.

   b. An acetate/citrate buffer of pH 6.0 was made by dissolving 57 g of sodium acetate ($3H_2O$), 37.5 g of trisodium citrate ($2H_2O$), 5.5 g of citric acid (H_2O) and 385 ml of isopropanol in water and made up to 1 liter with water.

   Just before the start of each series of determinations, solutions 1(a) and 1(b) were mixed in the proportion of 1 volume of 1(a) to 4 volumes of 1(b) to give the oxidant solution (about 1.25 ml per analytical value required).

2. Ehrlich's Reagent Solution

   a. p-Dimethylamino-benzaldehyde was dissolved in 60% perchloric acid (S.G. 1.54) in the proportion of 2 g of aldehyde to 3 ml of acid.

   b. Isopropanol.

   Just before the start of each series of determinations, solutions 2(a) and 2(b) were mixed in the proportions of 3 volumes of 2(a) to 13 volumes of 2(b) to give a final volume of about 15 ml per analytical value required, allowing for manipulation.

3. Hydroxyproline Standard Solution

25 mg of trans-L-hydroxyproline was made up in 0.001 M HCl to minimize bacterial growth. The solution was refrigerated (4°C) and only portions taken for dilution were allowed to reach room temperature.

4. Hydroxyproline Standards

The stock solution was diluted (10 ml to 100 ml) such that 1 ml of diluted stock contained 10 ug hydroxyproline/ml distilled H_2O.)

TEST TUBE #	DILUTED STOCK (ml)	H_2O (ml)	Conc'n ug/ml	O.D.
Blank	-	1.0	0	
1	0.1	0.9	1.0	
2	0.25	0.75	2.5	
3	0.5	0.5	5.0	
4	0.75	0.25	7.5	
5	1.0	-	10.0	

PROCEDURES

I. Total Hydroxyproline

Steps

1. Frozen tissue was weighed and recorded.
2. Tissue and 10 ml 6 N HCl was added to 50 ml hydrolysis bottle.

Autoclave

3. Samples were sealed (rubber stoppers and metal caps) and autoclaved at 130°C for 3 hours, (hydrolysate seems to remain stable for several weeks if left sealed).

Neutralize

4. 5 ml hydrolysate was added to 50 ml Erlenmeyer flask.
5. 3 drops congo red added (hydrolysate turns dark blue).
6. Titrate with 3 N NaOH until pH of 6-7 (hydrolysate turns to orange-yellow colour). This volume is recorded (usually 10 ml).
7. Add Norit (Fisher Norit A-alkaline), to decolourize. Let stand 5 minutes.
8. Filter (Fisher Whatman filter #1).

Colour Reaction (Bergman and Loxley, 1963).

9. Pipet - 1 ml distilled water
   - 1 ml of standards
   - 1 ml of neutralized sample, into individual 20 x 150 mm test tubes. All samples done in triplicate.

10. To each test tube, add the following in sequence:
    - 2 ml isopropanol and mix
    - 1 ml oxidant solution - mix, and let stand 4 minutes
    - 13 ml Ehrlich's reagent - mix.
11. Place tubes into water bath (60°C) for 25 min.
12. Cool tubes quickly in cold tap water.
13. Dilution step (isopropanol) was omitted and spectrophotometric readings were determined within 30 min.

II. <u>0.45 M NaCl Extraction</u> (0.45 M 26.301 gm NaCl/litre H_2O)

14. Frozen tissue was weighed and recorded.
15. Transfer to unbreakable centrifuge tube. Wash homogenization flask twice with 3 ml then 2 ml of 0.45 M NaCl and add to centrifuge tube (contains 20 ml 0.45 M NaCl plus all homogenized tissue).
16. Cover and leave in fridge overnight.
17. Centrifuge for 1 hour in refrigerated centrifuge.

Soluble Fraction

18. Pipet 15 ml supernatant into 100 ml hydrolysis bottle.
19. Add 10 ml 6 N HCl for hydrolysis and autoclave at 130°C for 3 hours.
20. Neutralize (steps 4 - 8).
21. Colour reaction (steps 9 - 13).

Insoluble Fraction

22. Decant remainder of supernatant and add 5 ml 6 N HCl to centrifuge tube containing solid pellet.
23. Pour into 100 ml hydrolysis bottle.
24. Wash centrifuge tube with another 5 ml 6 N HCl and add to hydrolysis bottle.
25. Continue procedure (steps 3 - 13).

Spectrophotometric Estimation of Hydroxyproline

26. O.D. readings taken at 558 nm.

APPENDIX C

TABLE V

COMPUTED CELL MEANS MATRIX OF
BODY WEIGHTS (g) OF MALE WISTAR RATS
AS A FUNCTION OF AGE (WKS) AND
ACTIVITY PATTERN

		FACTOR B ACTIVITY PATTERN		
		Control Sedentary CS	Endurance Control EC	Sprint Interval SI
FACTOR A AGE (WKS)	15	407.40±9.6 $n_{11}=10$	349.22±27.3 $n_{12}=9$	368.38±15.9 $n_{13}=8$
	25	504.50±9.6 $n_{21}=11$	451.14±8.03 $n_{22}=7$	488.83±10.4 $n_{23}=9$
	32	508.29±19.5 $n_{31}=7$	447.00±35.3 $n_{32}=6$	479.67±16.8 $n_{33}=6$

TABLE VI

SCHEFFÉ MULTIPLE COMPARISONS OF
ACTIVITY PATTERN MAIN EFFECTS
OF BODY WEIGHTS (g)
IN YOUNG AND ADULT MALE RATS

I	J	Contrast	F Ratio	Probability
CS	EC	57.6	8.01	0.00008
CS	SI	27.8	1.91	0.1570
EC	SI	-29.8	1.97	0.1481

TABLE VII

SCHEFFE MULTIPLE COMPARISONS OF
AGE (WKS) MAIN EFFECTS
OF BODY WEIGHTS (g)
IN YOUNG AND ADULT MALE RATS

I	J	Contrast	F Ratio	Probability
15	25	-106.5	30.3	0.0000
15	32	-103.3	23.9	0.0000
25	32	3.2	0.02	0.9780

TABLE VIII

SCHEFFE MULTIPLE COMPARISONS OF
AGE (WKS) MAIN EFFECTS
OF TOTAL COLLAGEN IN ACHILLES TENDON
AS INDICATED BY HYDROXYPROLINE CONCENTRATIONS
(ug/mg STORED WEIGHT)
OF YOUNG AND ADULT MALE RATS

I	J	Contrast	F Ratio	Probability
15	25	23.7	1.35	0.267
15	32	43.2	3.99	0.02
25	32	19.6	0.76	0.473

TABLE IX

SCHEFFE MULTIPLE COMPARISONS OF
ACTIVITY PATTERN MAIN EFFECTS
OF TOTAL COLLAGEN IN ACHILLES TENDON
AS INDICATED BY HYDROXYPROLINE CONCENTRATIONS
(ug/mg STORED WEIGHT)
OF YOUNG AND ADULT MALE RATS

I	J	Contrast	F Ratio	Probability
Control-Sedentary	Endurance Continuous	10.4	0.26	0.7705
CS	Sprint Interval	2.45	- 0.13	0.9875
EC	SI	8.0	0.13	0.8798

TABLE X

SCHEFFE MULTIPLE COMPARISONS OF
AGE (WKS) MAIN EFFECTS
OF INSOLUBLE COLLAGEN IN ACHILLES TENDON
AS INDICATED BY HYDROXYPROLINE CONCENTRATION
(ug/mg STORED WEIGHT)
OF YOUNG AND ADULT MALE RATS

I	J	Contrast	F Ratio	Probability
15	25	6.9	- 7.92	0.9239
15	32	29.1	- 1.29	0.2876
25	32	22.2	- 0.77	0.4678

TABLE XI

SCHEFFE MULTIPLE COMPARISONS OF
ACTIVITY PATTERN MAIN EFFECTS
OF INSOLUBLE COLLAGEN IN ACHILLES TENDONS
AS INDICATED BY HYDROXYPROLINE CONCENTRATION
(ug/mg STORED WEIGHT)
OF YOUNG AND ADULT MALE RATS

I	J	Contrast	F Ratio	Probability
Control-Sedentary	Endurance Continuous Sprint	- 30.9	- 1.52	0.2287
CS	Interval	- 0.8	-11.30	0.9988
EC	SI	30.1	1.30	0.2823